Environ Earth Sci (2010) 61:1207–1213
DOI 10.1007/s12665-009-0444-3

ORIGINAL ARTICLE

Influence of humic acids on the accumulation of copper and cadmium in *Vallisneria spiralis* L. from sediment

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Received: 3 August 2009 / Accepted: 29 December 2009 / Published online: 3 February 2010

Abstract Physiological responses and metal accumulation in *Vallisneria spiralis* L. exposed to copper and cadmium contaminated sediment were examined at different metal concentrations and the influence of humic acids on copper and cadmium accumulation was also studied. The plants of *V. spiralis* accumulated high amount of copper and cadmium. The maximum accumulation of 396 and 114 mg kg^{-1} DW copper were found in the roots and shoots, respectively, at 614 mg kg^{-1} DW after 21 days' copper exposure; they were 63.8 and 48.0 mg kg^{-1} DW for cadmium at 88.69 mg kg^{-1} DW. The plants showed decrease in chlorophyll content with the increasing concentration of copper/cadmium in sediment. With addition of humic acids from 3.09 to 7.89 g kg^{-1} DW, both copper and cadmium accumulation in *V. spiralis* were significantly inhibited ($p < 0.01$). The cadmium concentrations of roots and shoots of plant decreased 26.4–50.3 and 14.3–33.0% under cadmium treatments, respectively; copper accumulation decreased much more with 44.0–77.0 and 35.0–62.7%, respectively. It was concluded that *V. spiralis* appeared to be an ideal candidate for the phytoremediation of copper and cadmium polluted sediments, and humic acids had an important role in regulating copper and cadmium bioavailability and toxicity in sediments.

Keywords Phytoremediation · Heavy metals · In situ · Submerged macrophyte · Humic acids

Introduction

Many urban lakes and rivers have been loaded with considerable amounts of toxic heavy metals from point and non-point sources due to the human activity (Kähkönen et al. 1997). Heavy metals released into aquatic systems are generally bound to particulate matter, which eventually settle down and become incorporated into sediments. Surface sediment therefore is the most important reservoir or sink of metals and other pollutants in waters. Heavy metals usually possess significant toxicity to aquatic organisms, and then affect human health through food chain. Therefore, application of remediation technologies fitting for polluted sediment becomes necessary.

In recent years, there has been an ever-increasing interest in the study of metal-accumulating plants for environmental remediation application, termed as phytoremediation. This technology is widely viewed as an ecologically responsible alternative to the environmentally destructive chemical remediation methods currently practiced (Meagher 2000). One method of phytoremediation is phytoextraction, which uses metal-accumulating plants to remove pollutants from contaminated soils by concentrating them in the harvestable aboveground parts (Salt et al. 1998). Previous studies have shown that some aquatic macrophytes can accumulate large amounts of heavy metals in their organs (Deng et al. 2004; Kamal et al. 2004). In addition, some scientists have also studied the phytoremediation of aquatic macrophytes for contaminated sediments (Audet and Charest 2007). The use of aquatic plants to remedy heavy metal polluted sediments has been

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shown some excellent effects in some shallow rivers, lakes and wetlands (Peng et al. 2009).

The submerged macrophytes are particularly useful in the abatement and monitoring of heavy metals. They do not migrate and attain equilibrium with their surroundings within a short period (Gupta and Chandra 1998). These plants have been recommended for use in remediation of toxic metals from waste water (Rai et al. 1995). Among submerged macrophytes, *Vallisneria spiralis* Linn. (family Hydrocharitaceae; common name: ‘flat grass’, ‘noodle grass’ and ‘channel grass’) is widely distributed in shallow-water lakes in Asia and growing well in good transparence, deep-thick silt and slow-flow water areas (Sun 1992). *V. spiralis* plays a significant role in decreasing eutrophication of water body for its productivity and disposing industrial wastewater for its adaptability and well potential pollutant absorption (Xian et al. 2006), and it has also been shown to accumulate significantly high amount of heavy metals from water bodies, including copper and cadmium (Sinha et al. 1994; Gupta and Chandra 1998; Sinha et al. 2002), and its phytoremediation effect of heavy metals from water is well documented (Rai and Tripathi 2009). However, little is known about the ability of this plant to accumulate toxic metals from sediments, especially when heavy metals are applied in moderately high concentration. One of the objectives of the present investigation was to study the heavy metal accumulation in *V. spiralis* from sediment and the effects of heavy metal concentration on the accumulation.

Humic acids make up an important part of sediment organic matter. The ecochemical relevance of humic acids is mostly discussed with respect to their capability to bind or integrate pollutants like organic xenobiotics and heavy metals, consequently decreasing the bioavailability and toxicity of these pollutants (Haitzer et al. 1998). The binding strengths between heavy metals and humic molecules may vary, depending upon the nature of pollutants and on the general composition of the aquatic environment (Misra et al. 1996); their binding capacity affects the fate of metal ions and plays an important role in their mobility (Stevenson 1994). Information regarding humic acid influence heavy metals to submerged macrophyte is scanty, so another objective of this study was to determine the interactive effects of copper/cadmium and humic acids on metals accumulation in *V. spiralis*.

Materials and methods

Plant material

V. spiralis was collected from Shikesong River (114°19′E, 31°09′–31°10′N), in Wuhan, China. The collected plants were then cultivated with tap water in a large tank in a greenhouse under the natural day–night cycle. After the plants were grown for more than 3 weeks, the uniform-sized plants were selected for experimental purpose.

Experimental design

Pot experiments were designed. Each china tanks (100 L volume with 80 cm height) as the experimental apparatus was filled with 5 kg air-dried sediment. The sediment was surface sediment, dredged from Yuehu Lake (114°14′–114°15′E, 30°33′N), in Wuhan, China. Due to receiving the wastewater in long time, the sediment contained organic and inorganic pollutants. All sediment samples were analyzed in triplicate for general sediment properties and total metal content (Table 1).

Standard solutions of copper [$CuCl_2 \cdot 2H_2O$] and cadmium [$CdCl_2 \cdot 2.5H_2O$] (AR) were added to the sediments. Four copper levels (214, 314, 414, 614 mg kg^{-1} DW) and four cadmium levels (8.69, 28.69, 48.69, 88.69 mg kg^{-1} DW) were arranged in two experiments. Experiment 1 (with eight treatments) was intended to evaluate the effects of different metal levels on metal accumulation when plants were separately exposed to copper and cadmium (Table 2).

To study on the influence of humic acids on heavy metal accumulation, humic acids isolated from weathered coal

Table 1 The properties and metal concentrations of the sediment used in the pot experiments

pH (1:1 soil/1 M KCl ratio)	7.3 ± 0.12
Eh (mv)	−114 ± 5.77
CEC (cmol kg^{-1})	111 ± 0.02
OM (%)	10.6 ± 0.31
$CaCO_3$ (%)	1.77 ± 0.08
Humic acids (g kg^{-1})	3.09 ± 0.03
Cu (mg kg^{-1} soil DW)	214 ± 1.85
Cd (mg kg^{-1} soil DW)	8.69 ± 0.004

All the values are mean of three replicates ± SD

pH-H_2O actual sediment acidity, *Eh* sediment redox potential, *$CaCO_3$* sediment carbonate content, *OM* organic matter content, *CEC* cation exchange capacity

Table 2 Experimental design showing the metal concentrations used in the experiment on metal accumulation

Set	Metal		Concentration in sediments (mg kg^{-1} DW)			
1	Cu	Adding	0	100	200	400
		Final	214	314	414	614
2	Cd	Adding	0	20	40	80
		Final	8.69	28.69	48.69	88.69

Table 3 Chemical properties of humic acid isolated from weathered coal

Item	Content
C (%)	68.75[a]
H (%)	2.73[a]
N (%)	1.28[a]
O + S (%)	28.23[a]
Total acidity (mmol g^{-1})	6.24[a]
$COOH^-$ (mmol g^{-1})	4.51[a]
Phenolic OH^- (mmol g^{-1})	1.78[a]

[a] Results presented on a dry and ash free basis

(Taiyuan Mapon Humic Acids Development Co., Ltd., Shanxi, China) was used. The chemical properties of humic acids were listed in Table 3. The sediment was added with humic acids 24 g aiming to increase the content of humic acids in sediment from 3.09 to 7.89 g kg^{-1} DW and to simulate a high organic matter condition. The copper/cadmium treatments were similar to those in sediments without humic acids addition as Experiment 1 (Table 2). Experiment 2 (with eight treatments) investigated the effect of humic acids on metal accumulation (Table 4).

There were three replicates per each treatment. In order to prevent heavy metals from diffusing into water, a thin untreated sediment (1 cm) was laid on the treated sediment surface. Then, All tanks were flooded with 60 L tap water. Each tank was planted with 30 healthy seedlings of *V. spiralis* with approximately 75 g fresh weight. During the experiment, the light condition was natural (14 h:10 h day/night), and the mean temperature was 34°C during the day and 29°C at night. The relative humidity was approximately 60–70%.

Sediment characterization

The pH of sediment was measured in 1:1 soil water suspension using Orion pH meter (Model 420); the redox potential of sediment was also determined using a redox potential meter (Model 150). The cation exchange capacity of sediment was determined by first saturating the soil matrix with NH_4^+, then desorbing the NH_4^+ by K^+ and measuring the quantity of the NH_4^+ in the leachate (Van Ranst et al. 1999). Organic matter content was determined by weighing oven-dried soil (1.00 g) in a dry crucible; the weighed sample was then placed in a muffle furnace (375°C) and left overnight. The sample was cooled in a desiccator and re-weighed (Hartley and Lepp 2008). Total carbonate content present in sediment was determined by adding a known excess quantity of sulphuric acid and back-titrating the excess with sodium hydroxide (Van Ranst et al. 1999). Humic acids were extracted with 1 M sodium hydroxide solution according to the method described by Jesenak (1982). Total metal contents [samples digested with conc. HCl + conc. $HClO_4$ (4:1, v/v) (McGrath and Cunliffe 1985)] were measured by inductively coupled plasma–optical emission spectrometry, ICP–OES (Intrepid XSP Rasial ICP–OES, Thermo., USA) for copper and cadmium.

Chlorophyll determination

Total chlorophyll was analyzed at 21 days of plant growth before harvest. Prior to the extraction, fresh shoot samples were cleaned with deionized water. Chlorophyll extraction was carried out on fresh and fully expanded shoot materials. Shoot sample was extracted in ethanol:acetone (1:1, v/v) under the dark condition. The absorbance at 663 and 645 nm was measured using a UV/Visible spectrophotometer (Lengguang, UV-754N, China). Chlorophyll concentrations were calculated using the equation described by Inskeep and Bloom (1985).

Plant harvest and analysis

All the plant individuals were harvested after 21 days' cultivation. The sediment attached upon the roots was carefully removed by slowly running tap water. The plants were freshly weighed, and shoots and roots were further separated. Then every part was oven-dried at 70°C to constant. The dried materials were weighed and powdered for metal analysis. The powder of plant was digested by [conc. HNO_3 + conc. $HClO_4$ (4:1, v/v)] and metals (copper and cadmium) were analyzed by inductively coupled plasma–optical emission spectrometry, ICP–OES (Intrepid XSP Rasial ICP–OES, Thermo., USA) (Page et al. 1982).

Table 4 Experimental design showing the metal and humic acid concentrations used in the experiment on influence of humic acids on metal accumulation

Metal and humic acids		Concentration in sediments (mg kg^{-1} DW)			
Cu + HA	Adding	0 + 4.8	100 + 4.8	200 + 4.8	400 + 4.8
	Final	214 + 7.89	314 + 7.89	414 + 7.89	614 + 7.89
Cd + HA	Adding	0 + 4.8	20 + 4.8	40 + 4.8	80 + 4.8
	Final	8.69 + 7.89	28.69 + 7.89	48.69 + 7.89	88.69 + 7.89

Statistical analyses

All the data were the mean value of three samples in each tank. The mean values of metal accumulation were calculated and subjected to analysis of variance (ANOVA) using two-way ANOVA and least significant difference (LSD) methods after analysis as the homogeneity of variance. All statistical analyses were performed in SPSS 13.0 (SPSS Inc. Chicago, IL, USA) at 0.05 level.

Results

Chlorophyll content

The data presented in Table 5 showed the effects of different concentrations of copper without/with addition humic acids on the content of chlorophyll in *V. spiralis*. Chlorophyll contents decreased significantly with the increasing copper concentration of copper in the sediment. Chlorophyll a of *V. spiralis* increased significantly with humic acids addition ($p < 0.05$) after adding humic acids at 414, 614 mg kg^{-1} DW, while chlorophyll b content was hardly affected except at 314 mg kg^{-1} DW ($p > 0.05$).

Chlorophyll contents in *V. spiralis* decreased significantly with the increasing concentration of cadmium in sediment, too (Table 6). The addition of humic acids to the sediment increased chlorophyll a significantly at 48.69 mg kg^{-1} DW ($p < 0.05$), while there was no difference on chlorophyll b between control and humic acids addition after 21 days ($p > 0.05$).

Metal accumulation by *V. spiralis*

Copper

The concentrations of copper in the roots and shoots markedly increased with the increasing concentration of copper in the sediment (Fig. 1a). The maximum accumulation of copper (396 mg kg^{-1} DW) was found in the roots at 614 mg kg^{-1} DW in sediments after 21 days. At the same concentration and treatment duration, the shoots showed much lower (114 mg kg^{-1} DW) accumulation.

The treatment with humic acids addition caused a significantly lower concentration of copper in the shoots at 314, 414 and 614 mg kg^{-1} DW copper compared to the treatments without addition of humic acids. Whatever adding humic acids or not, the concentrations of copper were significantly higher in roots than in shoots of *V. spiralis* at all treatments, and the copper concentrations in roots or shoots of *V. spiralis* was positively correlated with the copper concentration in sediments ($p < 0.01$).

Cadmium

The accumulation of cadmium in *V. spiralis* treated with cadmium and humic acids for 21 days were showed in

Table 5 Effect of copper and humic acids on chlorophyll content (mg g^{-1} FW) in *V. spiralis*

Metal conc. (mg kg^{-1} DW)	Chlorophyll *a*		Chlorophyll *b*	
	Control	Humic acids	Control	Humic acids
214	0.408 ± 0.013	0.423 ± 0.006	0.125 ± 0.012	0.145 ± 0.009
314	0.406 ± 0.050	0.423 ± 0.018	0.096 ± 0.011	0.168 ± 0.000**
414	0.322 ± 0.016	0.418 ± 0.032*	0.087 ± 0.008	0.110 ± 0.028
614	0.270 ± 0.037	0.346 ± 0.006*	0.077 ± 0.008	0.088 ± 0.001

All values are mean of triplicate ± SD

* $p < 0.05$; ** $p < 0.01$

Table 6 Effect of cadmium and humic acids on chlorophyll content (mg g^{-1} FW) in *V. spiralis*

Metal Conc. (mg kg^{-1} DW)	Chlorophyll *a*		Chlorophyll *b*	
	Control	Humic acids	Control	Humic acids
8.69	0.408 ± 0.013	0.423 ± 0.006	0.125 ± 0.012	0.145 ± 0.009
28.69	0.349 ± 0.034	0.351 ± 0.032	0.101 ± 0.010	0.114 ± 0.009
48.69	0.300 ± 0.009	0.356 ± 0.022*	0.081 ± 0.001	0.095 ± 0.006
88.69	0.253 ± 0.013	0.254 ± 0.014	0.077 ± 0.011	0.061 ± 0.005

All values are mean of triplicate ± SD

* $p < 0.05$

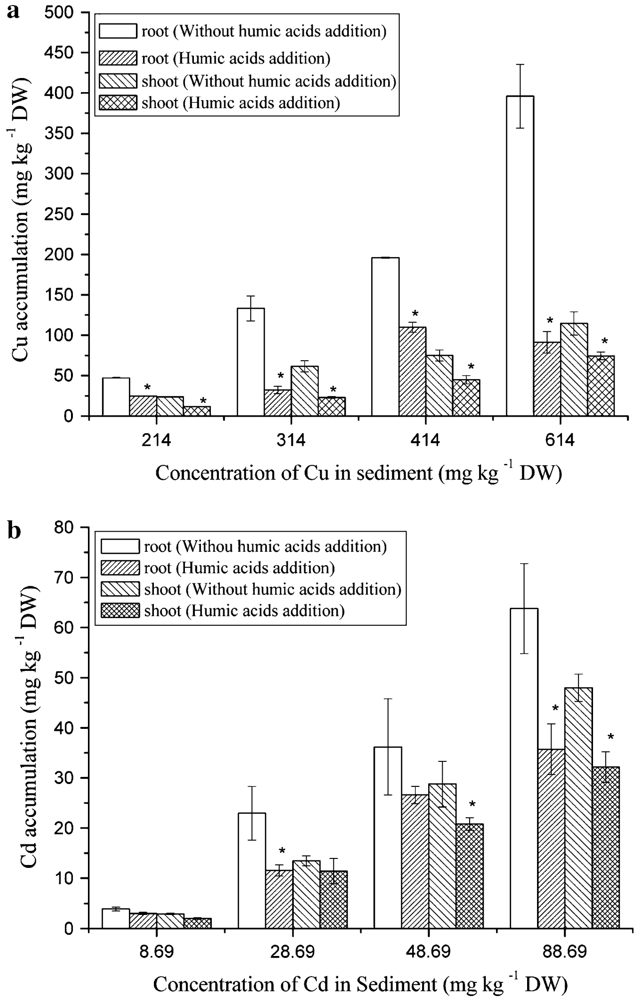

Fig. 1 Effect of heavy metals and humic acids application to sediment on heavy metals concentrations in *Vallisneria spiralis*. **a** Cu, **b** Cd. For comparing application humic acids with the control, *bars* marked with a (*) are statistically different ($p < 0.05$). The *bars* of column are the standard deviation of triplicates ($n = 3$)

Fig. 1b. The results showed that the metal accumulation increased significantly with the increasing concentration of cadmium in the sediment ($p < 0.01$), and cadmium accumulation in roots was significant higher than in shoots ($p < 0.01$). The plant accumulated highest amount of cadmium (63.8 and 48.0 mg kg^{-1} DW in roots and shoots, respectively) at treatment of 88.69 mg cadmium kg^{-1} DW.

Humic acids had significant inhibition effect on cadmium accumulation in both roots and shoots of *V. spiralis* ($p < 0.01$). For example, with the addition of humic acids, the plant accumulated 35.7 mg cadmium kg^{-1} DW in roots and 32.2 mg cadmium kg^{-1} DW in shoots, respectively, which were significantly lower than those treated with cadmium alone in the treatment 88.69 mg cadmium kg^{-1} DW ($p < 0.05$).

Discussion

Both copper and cadmium interact strongly with sulfhydryl groups inhibited the chlorophyll biosynthesis (Van Assche and Clijsters 1990). Degradation of the photosynthetic pigment chlorophyll under copper stress caused deficiency in light harvesting capacity and consequently decreased photosynthetic activity of the cell (Ouzounidou 1996), and similar result was found under cadmium stress (Rai et al. 2003).

The present study showed a reduction in chlorophyll content, the reduction being more at higher metal concentrations in sediment in situ, and confirmed the earlier study on submerged plants (Guilizzoni 1991). Sinha et al. (1994) also reported that the chlorophyll content in copper and cadmium-treated leaves of *V. spiralis* decreased with an increase in medium metal concentration.

St-Cyr and Campbell (2000) reported that metal concentrations in *Vallisneria* organs correlated closely with total metal concentrations in sediments, including copper and cadmium. In the present study, concentrations of copper and cadmium in *V. spiralis* roots were significantly related to these metal concentrations in the sediments ($p < 0.01$), respectively. In the case of shoots, significant relationships were also obtained ($p < 0.01$). Wang et al. (2009) also reported that the concentrations of cadmium in leaves and roots of *V. spiralis* increased with increasing cadmium concentrations in solutions. And the present study showed the same results in sediment in situ. Welsh and Denny (1979) reported that *V. spiralis* extracts nutrients mostly from the sediment which is translocated to the upper part. In the present study, copper concentrations were also higher in roots than those in the leaves of *V. spiralis*; the same was true for cadmium but with more variability.

V. spiralis showed a high accumulation of copper and cadmium from water (Sinha et al. 1994; Wang et al. 2009). It was also shown in the present study that *V. spiralis* could accumulate high content of metals (copper and cadmium) from the sediment when the exposure concentrations of copper were 214–614 mg kg^{-1} DW and cadmium were 8.69–88.69 mg kg^{-1} DW, respectively. It seemed to provide evidence that submerged macrophyte *V. spiralis* may be suitable for remediation of copper and cadmium polluted sediment.

Humic acids in sediment, frequently existing in considerable amounts in particle form, play an important role in heavy metal transformation. In the present study, the addition of humic acids resulted in a decrease of metal uptake capacity. The cadmium concentrations in roots and shoots of plant decreased 26.4–50.3 and 14.3–33.0% under cadmium treatments, and copper accumulation decreased much with 44.0–77.0 and 35.0–62.7% for roots and shoots, respectively.

Several studies reported on the decreases in metal uptake and toxicity caused by the addition of humic acids. The humic acids decreased uptake of copper have been observed (Inaba and Takenaka 2005; Remon et al. 2005). Bunluesin et al. (2007) reported that the accumulation of cadmium and zinc to submerged macrophyte *Ceratophyllum demersum*, was significantly reduced in the presence of humic acids. In addition, humic acids decreased lead uptake by *Lemna minor* and brought about increases in total chlorophyll content, growth rate and multiplication rate compared with the treatment with lead alone (Kruatrachue et al. 2002). Hence, humic acid could reduce metal uptake and toxicity in some species.

Addition of humic acids to sediment decreased the metal accumulation in *V. spiralis* from sediment. It indicated that humic acids resulted in decreasing concentrations of bioavailable metal forms, the free metal ions. Metal-binding by humic acids in sediment has been shown to affect the mobility of metals in the environment. Addition of humic acids reduced the extractability of the soluble and exchangeable forms of metals, including copper and cadmium. In the pH ranges of natural environments (7–8.5), humic acids carry a net negative charge, hence show high affinity for cationic metal species (Koukal et al. 2003). Koukal et al. (2003) also reported that cadmium free ion concentrations decreased in the presence of humic acids. Fan et al. (2007) also found the bioavailability of copper and cadmium in sediments was reduced in the presence of humic acid. In this study, the pH of the sediment with humic acids was 7.2, which was lower than that of the medium without humic acids (pH 7.4). The reduced bioavailability of metals in the presence of humic acids was believed to be due to competition for metals between complexing sites on humic acids and plants (Sunda and Huntsman 1998). Koukal et al. (2003) suggested that humic acids reduced metal toxicity in two different ways: (1) humic acids decrease the amount of free metal ions. Complexes of metal and humic acids are high molecular weight, relatively stable with regard to metal-exchange reactions and consequently the metals were less bioavailable. (2) humic acids adsorbed onto roots surfaces, shielded the cells from free metal ions. Fan et al. (2007) studied the change of species distribution of copper and cadmium in sediment through sequential extraction procedure and the results indicated that humic acids could promote the transformation of heavy metals from easily bioavailable fractions to potential bioavailable fractions. In another word, humic acids could stabilize heavy metals and reduce their bioavailability.

Conclusions

V. spiralis could be a suitable plant for phytoremediation of metal polluted sediment in situ, as it accumulated significant amounts of copper and cadmium from different concentration of treatments during a period of 3 weeks. However, reduction in chlorophyll was also observed. Humic acids clearly decreased copper and cadmium bioavailability and toxicity in *V. spiralis* due to the formation of complexes of humic acids with metal ions.

Acknowledgments This work was supported by Project of National Major Program of Science and Technology (2008ZX07316-004) and the Key Project of Knowledge Innovation Program of Chinese Academy of Sciences (KZCX2-YW-426). The authors would like to thank Dr. Man He at Wuhan University for her contribution, as well as the other colleagues of the research group for their help during this work.

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